

QUANTITATIVE IMMUNOHISTOCHEMISTRY USING THE APERIO WHOLE SLIDE IMAGING SYSTEM EVALUATING ANGIOGENESIS AND HYPOXIA MARKERS IN PANCREATIC CARCINOMA MOUSE MODEL TREATED WITH VEHICLE CONTROL, E3330, AND A STAT 3 INHIBITOR

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Investigation of the signaling pathways and molecular mechanisms that are major contributors to pancreatic tumor progression and its resistance to traditional therapies is lacking. Human apurinic endonuclease/redox factor 1 (APE/Ref-1) mediates repair of radiation-induced DNA lesions and regulates transcription via redox-based activation. Transcriptional factors HIF-1 α , NF κ B, and AP-1 are regulated by Ref-1 and are implicated in pancreatic tumor growth and the response to hypoxia. CD31 and CA IX (carbonic anhydrase) were biomarkers used in an in vivo study to evaluate the effectiveness of E3330, an APE 1 inhibitor, in a pancreatic mouse model. Immunostained slides were scanned using the Aperio automated whole slide scanning system (Scanscope CS) and were viewed using ImageScope™. Single fields of view from each WSDI measuring $\sim 10,000,000 \mu\text{m}^2$ and representing the whole area of the tumor were selected for analysis using the Aperio positive pixel algorithm.

The preclinical xenograft model evaluated human pancreatic carcinoma cell lines grown in NOD/SCID mice treated with the E3330 compound, a STAT 3 inhibitor, and an untreated vehicle control group. Immunohistochemistry (IHC) was used to predict effectiveness of treatment for pancreatic carcinoma based on CD31 and CA IX biomarker expression. IHC slides were quantified using both a traditional pathology hand count and the Aperio Imaging Analysis System. The positive pixel algorithm data closely mirrored the hand count for two biomarkers (CD31 and CA IX). In the E3330 treated group, the data showed CD31 (angiogenesis) was significantly knocked down with increased CA IX expression compared to the vehicle control. Hypoxia of the tumor cells was up in both treated groups. In summary, the Aperio imaging analysis system matched the hand count pathology data. The drug effects with E3330 exhibited both anti-angiogenesis and tumor hypoxia activity in the tumors.

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